



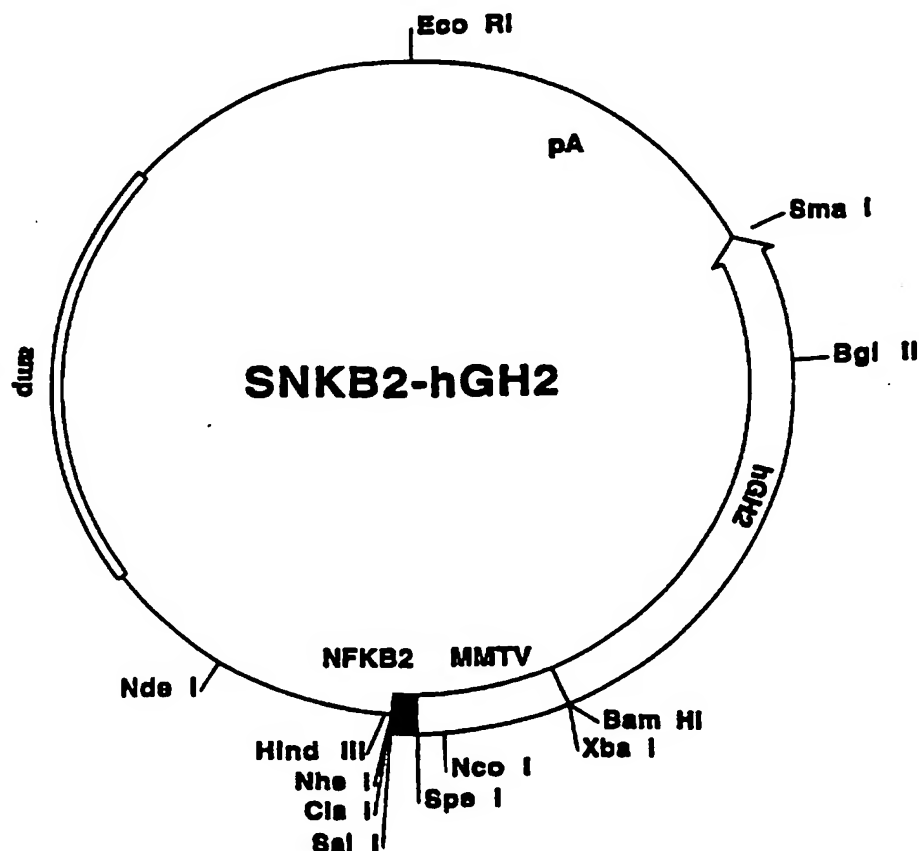
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C12N 5/10, 15/85, C12Q 1/68</b>	<b>A2</b>	(11) International Publication Number: <b>WO 96/17927</b> (43) International Publication Date: <b>13 June 1996 (13.06.96)</b>
<p>(21) International Application Number: <b>PCT/EP95/04819</b></p> <p>(22) International Filing Date: <b>5 December 1995 (05.12.95)</b></p> <p>(30) Priority Data: <b>9424497.7</b> <b>5 December 1994 (05.12.94)</b> <b>GB</b></p> <p>(71) Applicant (for all designated States except US): <b>KARO BIO AB [SE/SE]; Novum, S-141 57 Huddinge (SE).</b></p> <p>(72) Inventor; and (75) Inventor/Applicant (for US only): <b>NILSSON, Stefan [SE/SE]; Gunnebovagen 15, S-144 00 Ronninge (SE).</b></p> <p>(74) Agent: <b>DEAN, John, Paul; Withers &amp; Rogers, 4 Dyer's Buildings, Holborn, London EC1N 2JT (GB).</b></p>		<p>(81) Designated States: <b>AU, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</b></p> <p><b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i></p>

(54) Title: **REPORTER CELL LINE**

## (57) Abstract

The present invention provides a cell line including a first reporter gene arranged to express an assayable first gene product, expression of the first reporter gene being AP-1 mediated, and a second reporter gene arranged to express a second assayable gene product, expression of the second reporter gene being mediated by NFκB. Thus the present invention provides a convenient one cell line-based assay system which can be used to rapidly test a large number of compounds for potential anti-inflammatory activity. According to a further aspect of the invention there is provided a method of testing a compound for both AP1 and NFκB inhibitory activity, the method comprising providing cells in accordance with the first aspect of the invention, stimulating AP1 and NFκB activity in the cells and contacting the cells with the compound to be tested and monitoring expression of the first and second gene products wherein inhibition of AP1 expression of the first gene product, and inhibition of NFκB mediated expression of the second gene product is indicative that the compound has both AP1 and NFκB-inhibitory activity.



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**REPORTER CELL LINE**

5 This invention relates to a reporter cell line and is particularly, though not exclusively, concerned with a cell line useful for testing compounds as potential anti-inflammatory drugs.

10 Intracellular receptors and their ligands play key roles in various hormone dependent diseases such as cancers, osteoporosis and cardiovascular and inflammatory malfunctions. A number of pharmaceutical drugs that can modulate the function and activity of intracellular receptors have been developed and are today used for the treatment of major clinical indications: for example glucocorticoids as anti-inflammatory drugs, estrogen agonists, vitamin D and thyroid hormone for hormone replacement therapy, estrogen antagonists in the treatment of breast cancer and androgen antagonists  
15 in prostate cancer therapy.

However, for many indications there are either no hormonal drugs available or current drugs have undesired side effects due to poor receptor selectivity or tissue specificity. For example, Tamoxifen, a potent anti-estrogen used in adjuvant therapy in breast  
20 cancer, causes an increased frequency of primary endometrial cancer in long term treatment; or anti-inflammatory glucocorticoids for systemic treatment which frequently induce osteopenia.

0        Thus there is a need for safer drugs with an improved therapeutic profile, that is to say drugs having a more defined specificity and tissue selective agonist/antagonist activity.

5        Glucocorticoids exert profound effects on the inflammatory and immune responses. They affect growth, differentiation and function of a broad range of cells involved in these processes. The principal mechanism whereby they exert their powerful effects is through modulation of the transcription of specific sets of genes such as metalloproteases and inflammatory mediators such as the tumour necrosis factor TNF- $\alpha$ , and the interleukins IL-1,2,3, 5,6 and 8. Glucocorticoids inhibit transcription of these genes by interfering with the AP1 transcription factor, the heterodimeric complex formed by the c-fos and c-jun oncoproteins, (Angel *et al* (1987), Cell 49 729-739; Boumpas (1991) *et al* Clinical and Experimental Rheumatology 9, 413-423; Yang Yen *et al*, (1990) Cell 62,1205-1215, and references cited therein) and the NF $\kappa$ B transcription factor, the heterodimeric complex composed of two subunits, p50 and p65, involved in the expression of a number of genes including immunoglobulin  $\kappa$ , IL-2 receptor, and class 1

10        histocompatibility genes (Baeurle, (1991) Biochim. Biophys. Acta 1072 63-80; Stolpe *et al* (1994) J. Biol. Chem. 269:6185-6192; Ray and Prefontaine (1994) Proc. Natl Acad. Sci 91 752-756; Stein *et al* (1994) EMBO J. 12: 3879-3891; Caldenhoven *et al*. (1994) Mol. Endocrinol. (in press); and references cited therein).

15        Glucocorticoids also block the migration of leucocytes to the site of inflammation by having a direct inhibitory effect on the expression of adhesion molecules like ICAM and E-selectin (Caldenhoven *et al*. (1994) Mol. Endocrinol. (in press); and references cited

0       therein).

Experimental data suggests that the mechanism of AP1 and NF $\kappa$ B dependent transcription is suppressed by the hormone activated glucocorticoid receptor (GR) through the formation of a GR:AP1 and/or a GR: NF $\kappa$ B protein:protein complex, respectively. (Jonat *et al* (1990) *supra*; Yang Yen *et al* (1990) *supra*; Schüle *et al* 5 (1990) Cell 62, 1217-1226; Ray and Prefontaine (1994) *supra*; Caldenhoven *et al.* (1994)*supra*; and references cited therein).

10       It has been indicated from experimental data that the relative potency of synthetic and naturally occurring glucocorticoids as anti-inflammatory agents corresponds with their potency in inhibiting AP1-dependent transcription Jonat *et al* (1990); Boumpas *et al* (1991) *supra*; Yang-Yen *et al* (1990); and Schüle *et al* (1990) *supra*; unpublished results).

15       However, one of the most severe side effects of long term glucocorticoid treatment is steroid induced osteoporosis (in Osteoporosis 1990 edited by Christiansen C. and Overgaard K 1529-1538). A glucocorticoid induced negative calcium balance resulting in secondary hyperparathyroidism and inhibition of osteoblast precursor maturation is believed to be the major mechanisms of glucocorticoid induced bone loss (Hahn, T.J. 20 (1990), in Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism First edition, published by the American Society for Bone and Mineral Research p.158-162; Luckert B.P. and Raisz L.G., (1990) Annals. of Internal Medicine 112: 352-364;

0 and references cited therein).

The severe and negative side effects of existing, clinically used glucocorticoids points to the need for novel anti-inflammatory drugs with reduced toxicity.

5 Many of the large pharmaceutical companies have accumulated libraries exceeding 100,000 compounds which are of no market value until proven valuable for a clinical application.

10 Most of the drugs in current clinical use have been developed using classical techniques like *in vitro* binding to target receptors in tissue extracts or cells. Lead compound optimization has been performed by traditional medicinal chemistry. In addition, the lack of relevant *in vitro* assays for test of the biological activity of drugs have often necessitated large and extensive animal studies to prove their effects for the indicated clinical application. Initial screening of a large number of compounds on animals is  
15 time-consuming, expensive and is nowadays considered undesirable and, in certain cases, may give misleading results.

Recent advances in molecular biology and molecular endocrinology have made it possible to develop tools by which novel drugs can be discovered and evaluated in a  
20 rational way. It is today possible to do screening and evaluation of large compound libraries using genetically engineered, cell based transcription assays (reporter assays). This type of mechanistic *in vitro* assay not only discloses whether a compound interacts

0 with a specific hormone receptor but also the biological consequences of that interaction  
by indirectly determining the rate of transcription of a selected target gene (reporter  
gene).

According to a first aspect of the present invention there is provided a cell line  
5 including a first reporter gene arranged to express an assayable first gene product,  
expression of the first reporter gene being AP-1 mediated, and a second reporter gene  
arranged to express a second assayable gene product, expression of the second reporter  
gene being mediated by NF $\kappa$ B.

10 AP1 and/or NF $\kappa$ B activity in the cells may be stimulated and the cells subsequently  
contacted with a compound to be tested. Inhibition of either or both activities in the thus  
stimulated cells is indicative that the compound has at least potential anti-inflammatory  
activity.

15 Thus the present invention provides a convenient one cell line-based assay system which  
can be used to rapidly test a large number of compounds for potential anti-inflammatory  
activity.

Preferably, the cell line is derived from human, more preferably HeLa cells. The  
20 invention is not, however, limited to mammalian cells. For example, the cell line may  
be derived from yeast or insect cells.

0 One of the reporter genes may be arranged to express an alkaline phosphatase such as human placental alkaline phosphatase and the other reporter gene may be arranged to express human growth hormone. These gene products are preferred as they are readily assayed for, but other suitable reporter genes may be selected by the skilled worker.

5 According to a second aspect of the invention there is provided a method of testing a compound for both AP1 and NF $\kappa$ B inhibitory activity, the method comprising providing cells in accordance with the first aspect of the invention, stimulating AP1 and NF $\kappa$ B activity in the cells and contacting the cells with the compound to be tested and monitoring expression of the first and second gene products wherein inhibition of AP1  
10 expression of the first gene product, and inhibition of NF $\kappa$ B mediated expression of the second gene product is indicative that the compound has both AP1 and NF $\kappa$ B -inhibitory activity.

15 AP1 activity in the cells may be stimulated by the phorbol ester (TPA), epidermal growth factor (EGF), pyrrolidine dithiocarbamate (PDTTC) or N-acetyl-L-cysteine (NAC).

NF $\kappa$ B activity may be stimulated by TPA, TNF $\alpha$ , IL-1 $\beta$ , H<sub>2</sub>O<sub>2</sub> or any combination thereof.

20 According to a third aspect of the invention there is provided a method of testing a compound for AP1 inhibitory activity comprising providing cells in accordance with the



0 first aspect of the invention, stimulating AP1 activity in the cells, and contacting the cells with the compound to be tested and monitoring expression of the first and second gene products wherein inhibition of AP1-mediated expression of the first gene product and no effect or substantially no effect on the expression of the second gene product is indicative that the compound has AP1 inhibitory action.

5

According to a fourth aspect of the invention there is provided a method of testing a compound for NF $\kappa$ B inhibitory activity comprising providing cells in accordance with the first aspect of the invention, stimulating NF $\kappa$ B activity in the cells, and contacting the cells with the compound to be tested and monitoring the expression of the first and  
10 second gene products wherein inhibition of NF $\kappa$ B-mediated expression of the second gene product and no effect or substantially no effect on the expression of the first gene product is indicative that the compound has NF $\kappa$ B inhibitory activity.

15

AP1 activity or NF $\kappa$ B activity respectively may be stimulated in the methods according to the latter two aspects of the invention as described above in relation to the method in accordance with the second aspect of the invention.

20

The production of a cell line in accordance with the invention and its use to test compounds in methods in accordance with the invention will now be described, by way of example only, with reference to the accompanying Figures, Figs. 1 to 11 in which:

Fig. 1A shows the nucleotide sequence of the five AP1 responsive elements inserted in

0 tandem upstream of the mouse mammary tumour virus long terminal repeat (MMTV LTR) promoter and the gene encoding the secreted form of human placental alkaline phosphatase (ALP);

5 Fig 1B shows the ALP reporter vector 5APNT-ALP containing the nucleotide sequence of Fig 1A;

Fig 2A shows the nucleotide sequence of the two NF $\kappa$ B responsive elements inserted in tandem upstream of the mouse mammary tumour virus long terminal repeat (MMTV LTR) promoter;

10

Fig.2B shows the NF $\kappa$ B controlled reporter vector SNKB2-hGH2 encoding the human growth hormone polypeptide (hGH);

15

Fig. 3A illustrates the effect of dexamethasone on cells from a cell line in accordance with the invention in which AP-1 activity has been stimulated;

Fig 3B illustrates the effect of dexamethasone on cells from a cell line in accordance with the invention in which NF $\kappa$ B activity has been stimulated;

20

Fig 4 illustrates the effect of dexamethasone on cells from a cell line in accordance with the invention in which AP-1 and NF $\kappa$ B activity has been stimulated;

0        Fig 5 illustrates the effect of betamethasone on cells from a cell line in accordance with the invention in which AP-1 and NF $\kappa$ B activity has been stimulated;

Fig 6 illustrates the effect of beclomethasone on cells from a cell line in accordance with the invention in which AP-1 and NF $\kappa$ B activity has been stimulated;

5

Fig 7 illustrates the effect of aldosterone on cells from a cell line in accordance with the invention in which AP-1 and NF $\kappa$ B activity has been stimulated;

10

Fig 8 illustrates the effect of corticosterone on cells from a cell line in accordance with the invention in which AP-1 and NF $\kappa$ B activity has been stimulated;

Fig 9 illustrates the effect of cortisone on cells from a cell line in accordance with the invention in which AP-1 and NF $\kappa$ B activity has been stimulated;

15

Fig 10 illustrates the effect of cortexolone on cells from a cell line in accordance with the invention in which AP-1 and NF $\kappa$ B activity has been stimulated; and

Fig 11 illustrates the effect of RU486 on cells from a cell line in accordance with the invention in which AP-1 and NF $\kappa$ B activity has been stimulated.

20

#### 1        Generation of combined AP-1/NF $\kappa$ B reporter cell line

HeLa tk<sup>-</sup> cells were initially stably transformed using conventional techniques with a

0 reporter vector 5APNT-ALP comprising five AP1 response elements arranged in tandem and fused 5' to the core promoter sequences of the mouse mammary tumour virus long terminal repeat (MMTV LTR) and the gene encoding the secreted form of human placental alkaline phosphatase (ALP) (as shown in Figs 1A and B) whereby secretion of the ALP protein into the medium is indicative of AP-1 mediated transcription.

5 Fig. 1A shows the nucleotide sequence of the five AP1 responsive elements (over lined) inserted in tandem upstream of the TATA-box in the mouse mammary tumour virus long terminal repeat (MMTV LTR) promoter and the beginning of the gene encoding the secreted form of alkaline phosphatase (ALP) in plasmid 5APNT-ALP. The ATG (start  
10 codon) for ALP is underlined. The resulting cells were termed GRAP cells.

The level of ALP reporter protein expressed and secreted into the medium can be determined indirectly by a chemiluminescence assay. The facility to use a chemiluminescence based assay and the fact that the ALP is secreted into the culture  
15 medium, makes the ALP based reporter assay particularly convenient to use compared to intracellular reporters such as chloramphenicol acetyltransferase(CAT) and luciferase (Alam J. and Cook J.L.(1990) Analytical Biochemistry 188, 245-254). Additionally, the high sensitivity of the chemiluminescent ALP assay enables growth of cells and testing of compounds in 96-well microtiter plates.

20 Then a second, NF $\kappa$ B regulated reporter vector SNKB2-hGH2 shown in Fig 2B was introduced into the GRAP cells using conventional techniques for the stable transfection

0 of mammalian cells to produce a new cell line - termed NAP cells. The reporter vector SNKB2-hGH2 comprises a NF $\kappa$ B-regulated promoter (MMTV) fused to a reporter gene encoding human growth hormone (hGH). The hGH reporter protein is secreted into the cell culture medium like the ALP reporter protein. The level of NF $\kappa$ B-induced hGH expression is determined immunologically by a Delfia assay (Wallac OY, Finland).

5 Thus the NAP cells contain two exogenous transcription units whose reporter genes are transcriptionally induced by the two distinct signal-activated transcription factors AP1 and NF $\kappa$ B, respectively. The ALP transcription unit is controlled by, and responds to, the presence of elevated levels of the AP1 transcription factor induced by TPA, EGF,  
10 NAC or PDTC by increased ALP expression. On the other hand, the hGH transcription unit responds to signal activated NF $\kappa$ B resulting in an increase in hGH expression.

**2 Determining the effects of dexamethasone on the AP1-dependent ALP expression and NF $\kappa$ B dependent hGH reporter gene transactivation, in NAP cells**

15 The use of the NAP cells in determining the use of various compounds as anti-inflammatories can be demonstrated using the synthetic glucocorticoid dexamethasone.

Cells are cultured in MEM supplemented by 10%FCS, 1mM pyruvate and 1% non  
20 essential amino acids.

Before exposure to compounds, NAP cells are seeded in 96-well microtiter plates in

0 Ham's F12 ( without phenol red) supplemented with 0.25% bovine serum albumin and 50 $\mu$ g/ml gentamicin. The AP-1-dependent ALP reporter gene expression and the NF $\kappa$ B-dependent hGH reporter gene expression, respectively, was induced by 2  $\mu$ M phorbol ester TPA in Ham's F12 supplemented with 0.5% FCS and increasing concentrations of the synthetic glucocorticoid, dexamethasone.

5

The relative levels of ALP expressed were determined by a chemiluminescent assay as follows: a 10 $\mu$ l aliquot of the cell culture medium was mixed with 200 $\mu$ l of assay buffer (10mM diethanolamine pH 10; 1mM MgCl<sub>2</sub> and 0.5mM AMPPD) in accordance with the procedures of Tizard *et al* (1990) Proc. Natl Acad Sci.87 4514-4518) and Alksnis *et al* (1991) J. Biol. Chem. 266 10078-10085), in white microtiter plates and incubated at 10 37°C for 20 minutes before being transferred to a microplate format luminometer (Luminoskan Labsystems, Finland). The setting of the Luminoskan luminometer was integral measurement with 1 second reading of each well. The alkaline phosphatase activity is expressed in light units (LU).

15

hGH expression was monitored immunologically with the Delfia assay mentioned above.

20

The cells were treated with 2 $\mu$ M TPA to induce the heterodimeric fos/jun transcription factor (and therefore AP1 activity) and NF $\kappa$ B activity which was indirectly determined by an increased expression of ALP (Fig3A) and hGH (Fig3B), respectively. In the presence of increasing concentrations of dexamethasone, the AP-1 dependent ALP expression and the NF $\kappa$ B dependent hGH expression, respectively, were inhibited in a

0 dose dependent manner.

3 Determining the effects of various mineralocorticoids and glucocorticoids on  
the AP1-dependent ALP expression and NF $\kappa$ B dependent hGH reporter gene  
5 transactivation in NAP cells

Fig.s 4 to 10 illustrate the effect of various mineralocorticoids and glucocorticoids on  
NAP cells in which AP-1 and NF $\kappa$ B activity was previously stimulated as described  
above. It will be seen that each compound inhibited both AP-1 and NF $\kappa$ B dependent  
10 reporter gene expression to a greater or lesser effect. For example, the lower degree of  
inhibition shown in Figs 7 and 8 suggests that the mineralocorticoids tested, aldosterone  
and cortisone, are less likely to be good anti-inflammatories.

The cell line and the method of the present invention can be used to test a wide variety  
15 of compounds for AP1 and/or NF $\kappa$ B inhibitory activity and can be used in a compact  
convenient assay format.

## CLAIMS

0

1 A cell line including a first reporter gene arranged to express an assayable first gene product, expression of the first reporter gene being AP-1 mediated, and a second reporter gene arranged to express a second assayable gene product, expression of the second reporter gene being mediated by NF $\kappa$ B.

5

2 A cell line according to claim 1 in which AP1 and/or NF $\kappa$ B activity in the cells can be stimulated and the cells subsequently contacted with a compound to be tested.

10

3 A cell line according to claim 2 in which inhibition of either AP1 or NF $\kappa$ B activity in the thus stimulated cells is indicative that the compound has anti-inflammatory activity.

15

4 A cell line according claim 1 or 2 in which inhibition of both AP1 and NF $\kappa$ B activity in the thus stimulated cells is indicative that the compound has anti-inflammatory activity.

20

5 A cell line according to any preceding claim which is derived from human cells.

6 A cell line according to claim 5 which is derived from HeLa cells.

7 A cell line according to any preceding claim in which one of the reporter genes is arranged to express an alkaline phosphatase.



0

8 A cell line according to claim 7 in which the alkaline phosphatase is human placental alkaline phosphatase.

5

9 A cell line according to any preceding claim in which one or the other of the reporter genes is arranged to express human growth hormone.

10

10 A method of testing a compound for both AP1 and NF $\kappa$ B inhibitory activity, the method comprising providing cells from a cell line in accordance with any preceding claim, stimulating AP1 and NF $\kappa$ B activity in the cells and contacting the cells with the compound to be tested and monitoring expression of the first and second gene products, wherein inhibition of AP1-mediated expression of the first gene product, and inhibition of NF $\kappa$ B-mediated expression of the second gene product is indicative that the compound has both AP1 and NF $\kappa$ B -inhibitory activity.

15

11 A method according to claim 10 in which an indication that the compound has both AP1 and NF $\kappa$ B -inhibitory activity is indicative that the compound has an anti-inflammatory activity.

20

12 A method according to claim 10 or 11 in which AP1 activity in the cells is stimulated by the phorbol ester (TPA), epidermal growth factor (EGF), pyrrolidine dithiocarbamate (PDTC) or N-acetyl-L-cysteine (NAC).

0        13        A method according to claim 10, 11 or 12 in which NF $\kappa$ B activity is stimulated  
by TPA, H<sub>2</sub>O<sub>2</sub>, INF $\alpha$ , IL-1 $\beta$  or a combination of TPA and H<sub>2</sub>O<sub>2</sub>.

14        A method of testing a compound for AP1 inhibitory activity, the method  
comprising providing cells from a cell line in accordance with any one of claims 1 to 9,  
5        stimulating AP1 activity in the cells, and contacting the cells with the compound to be  
tested and monitoring expression of the first and second gene products wherein  
inhibition of AP1-mediated expression of the first gene product and no effect or  
substantially no effect on the expression of the second gene product is indicative that the  
compound has AP1 inhibitory action.

10

15        A method of testing a compound for NF $\kappa$ B inhibitory activity, the method  
comprising providing cells from a cell line in accordance with any one of claims 1 to 9,  
stimulating NF $\kappa$ B activity in the cells, and contacting the cells with the compound to be  
tested and monitoring the expression of the first and second gene products wherein  
15        inhibition of NF $\kappa$ B-mediated expression of the second gene product and no effect or  
substantially no effect on the expression of the first gene product is indicative that the  
compound has NF $\kappa$ B inhibitory activity.

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FIG. 1A

H i n d I I I	N h e I	C l a I	AP1	S a l I
AAGCTTGCTAGCATCGATTGAGTCAGATATGAGTCAGTATTTGAGTCAGGTCGACTGAGTC				
AGAAATGATATCTAATGAGTCAGACTAGTCCTCATGGAAATCTTATGTAAATGCTTATGTAAAC				
TATA				
CAAGATATAAAGAGTCCTGATTTTGTGAGTAAACTTGCACAGTCCTAACATTCACCTC				
TTGTGTGTTGTGCTGTTTCGCCATCCCGTTCTAGCATGCTGTGCTGCTGTGCTGCTG				
GGCCTGAGGCTACAGCTCTCCC				

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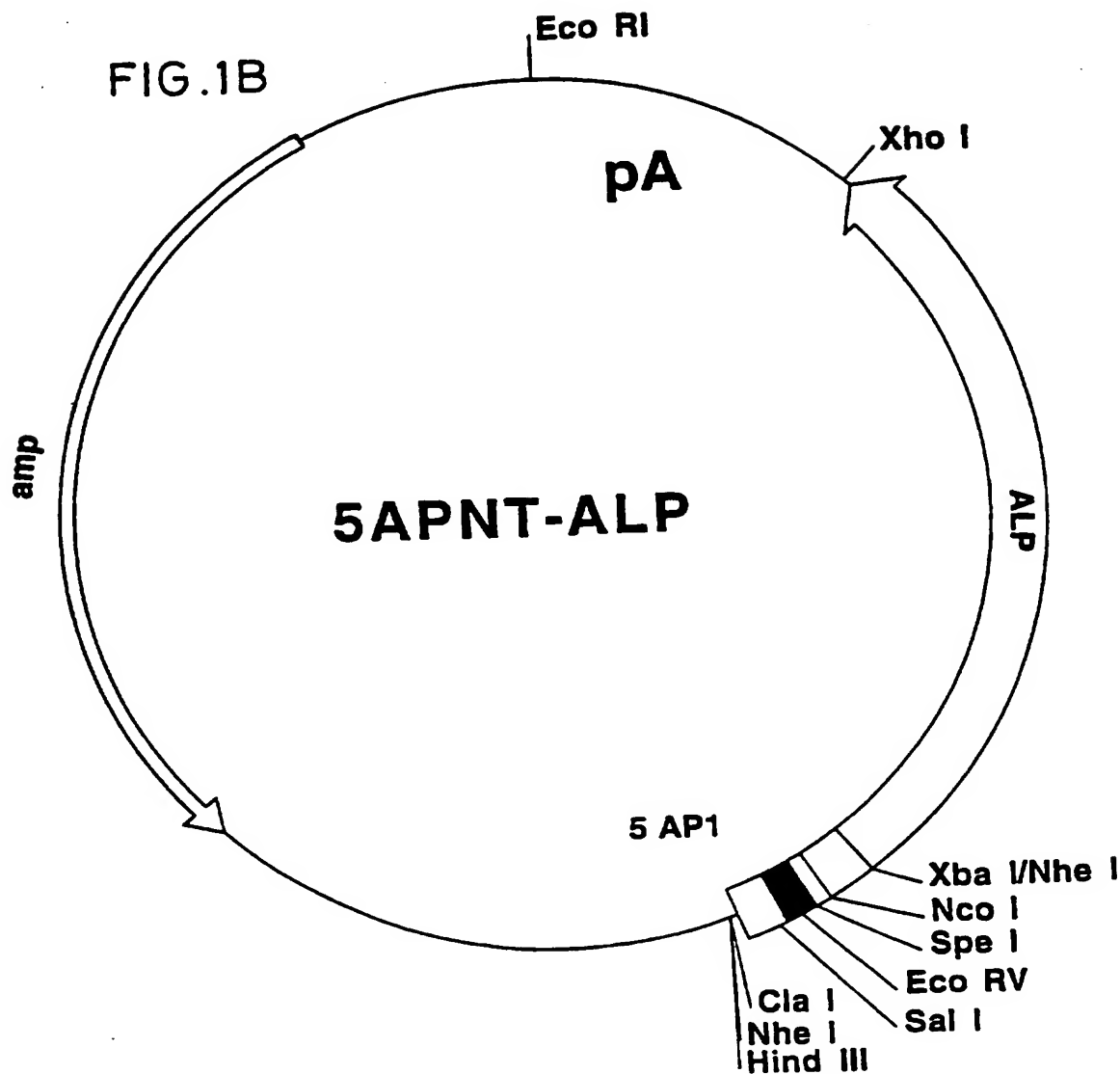
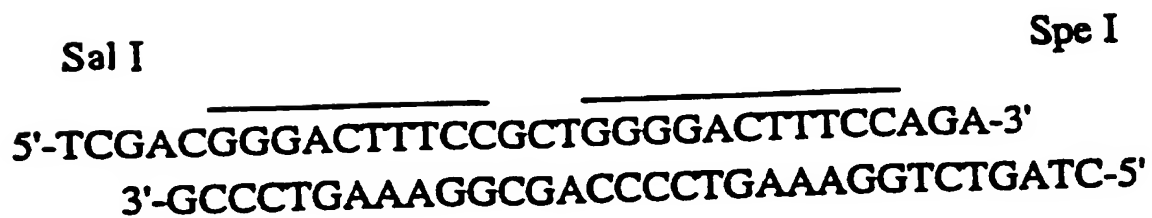


FIG.2A



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FIG. 2B

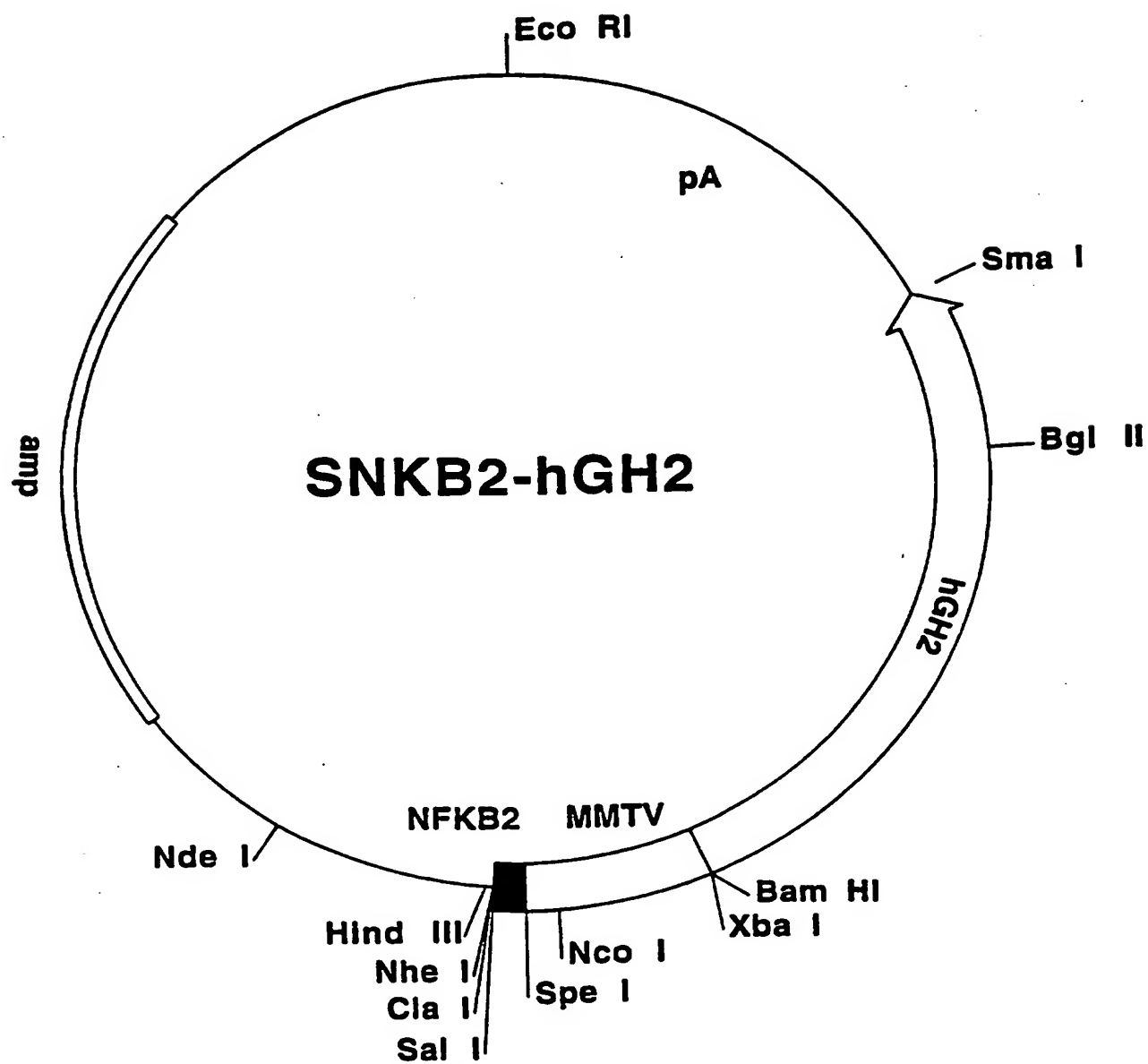


FIG. 3A

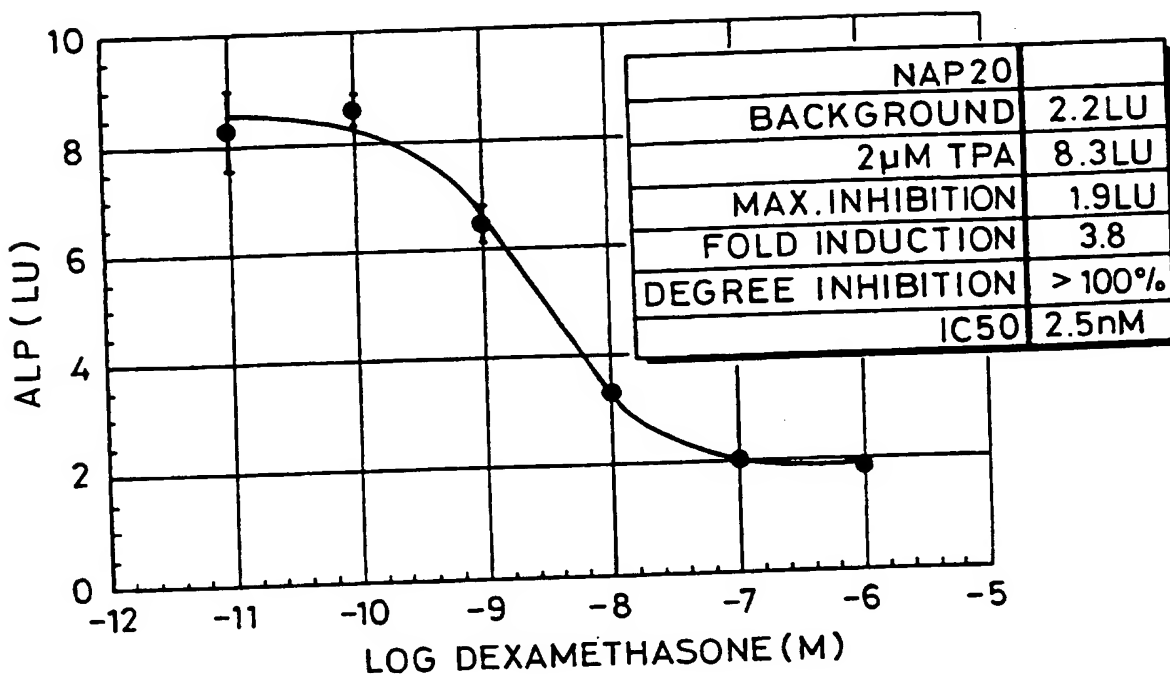


FIG. 3B

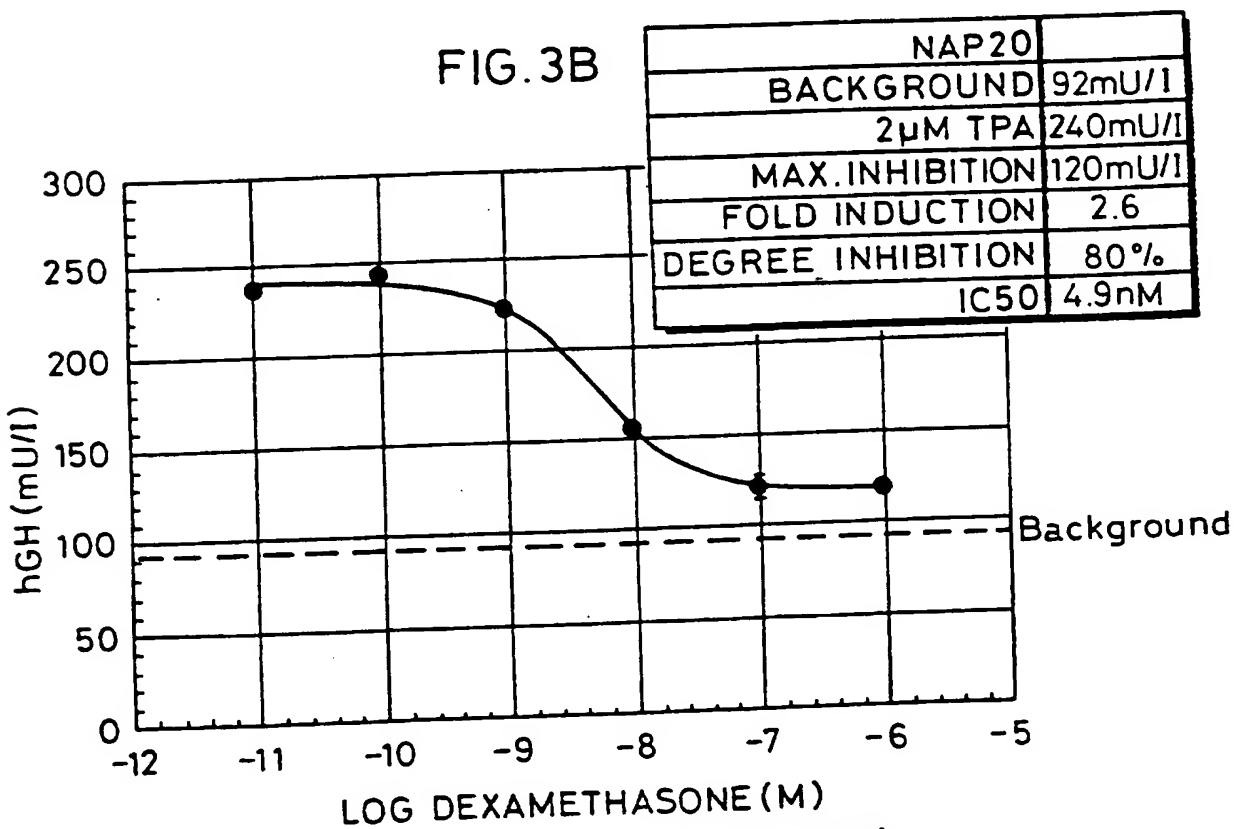


FIG. 4

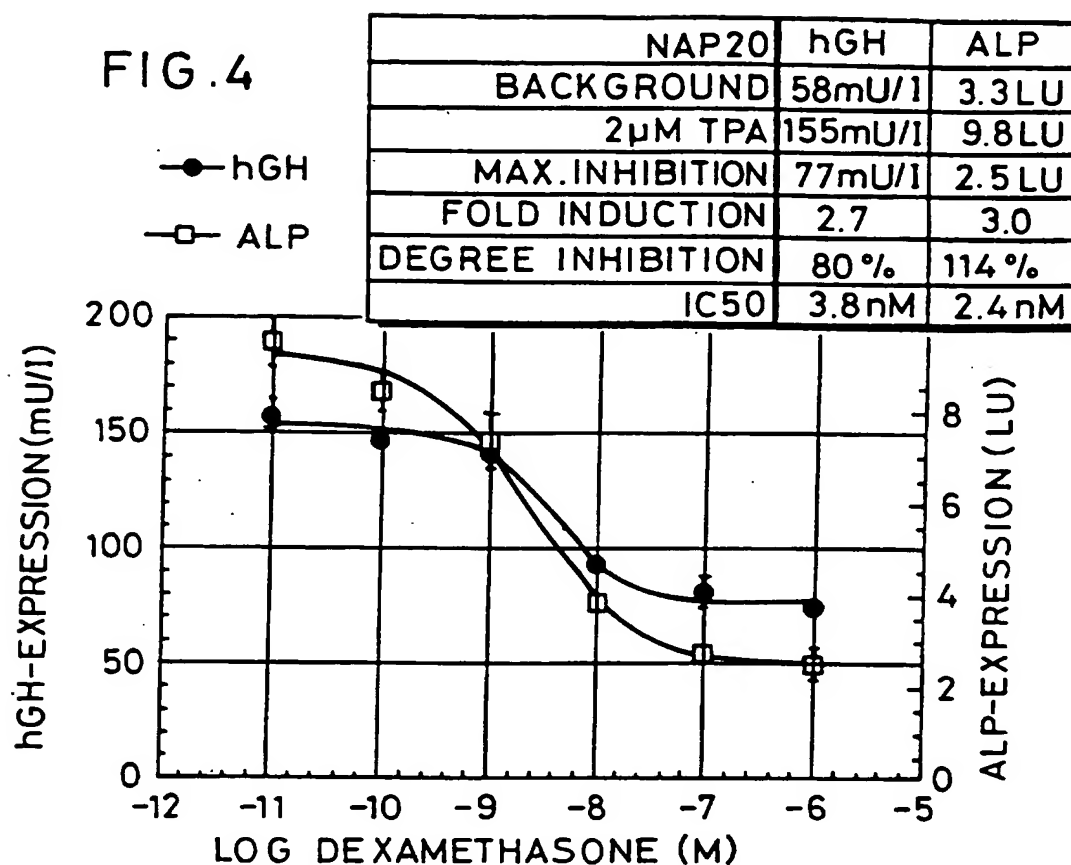
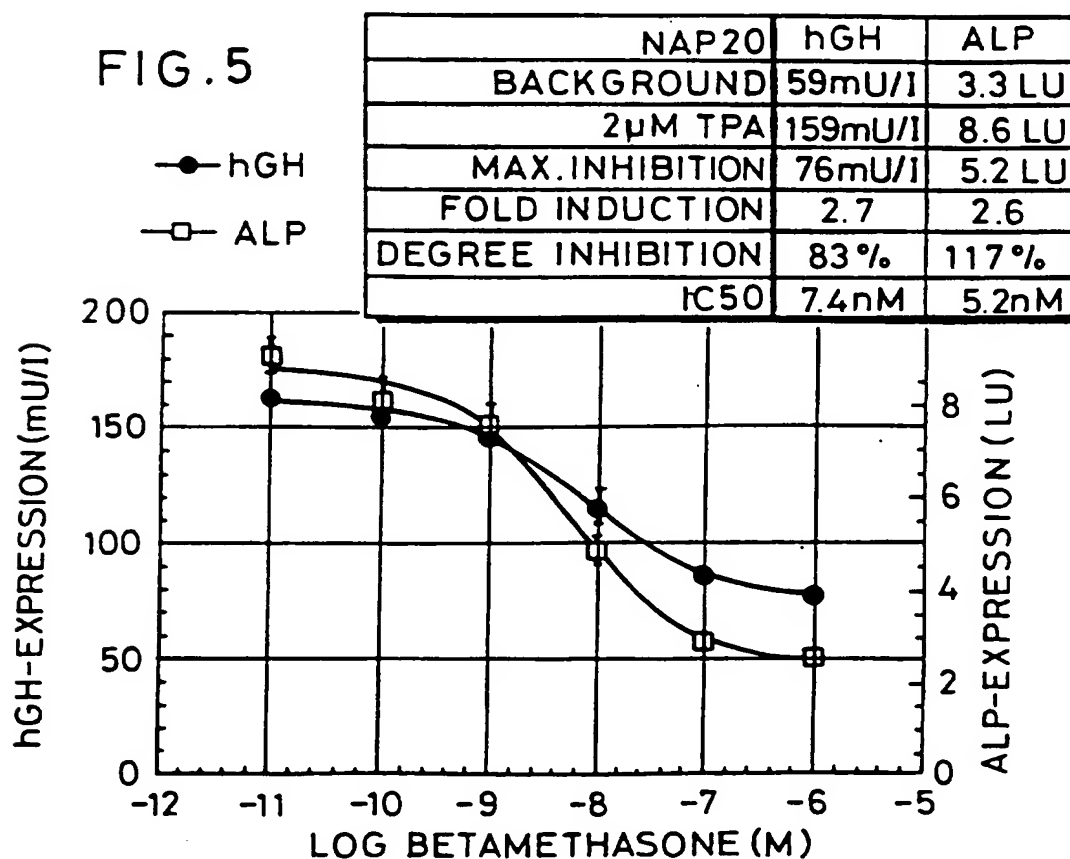


FIG. 5



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FIG. 6

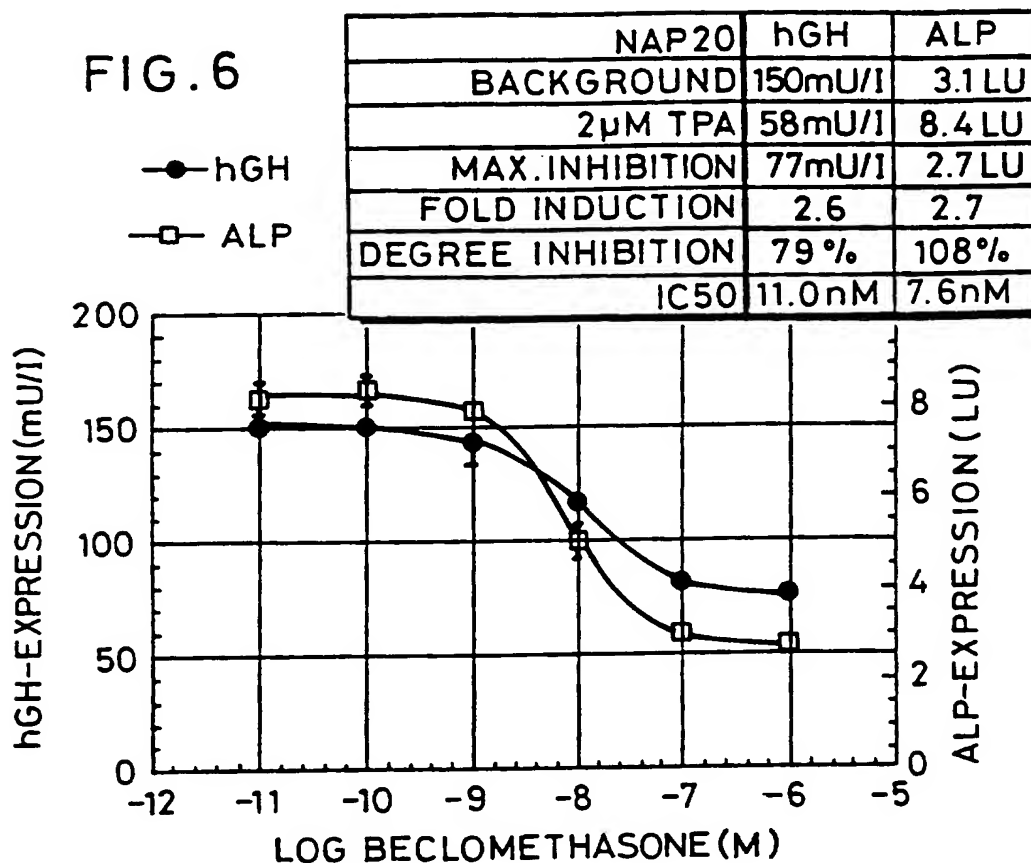
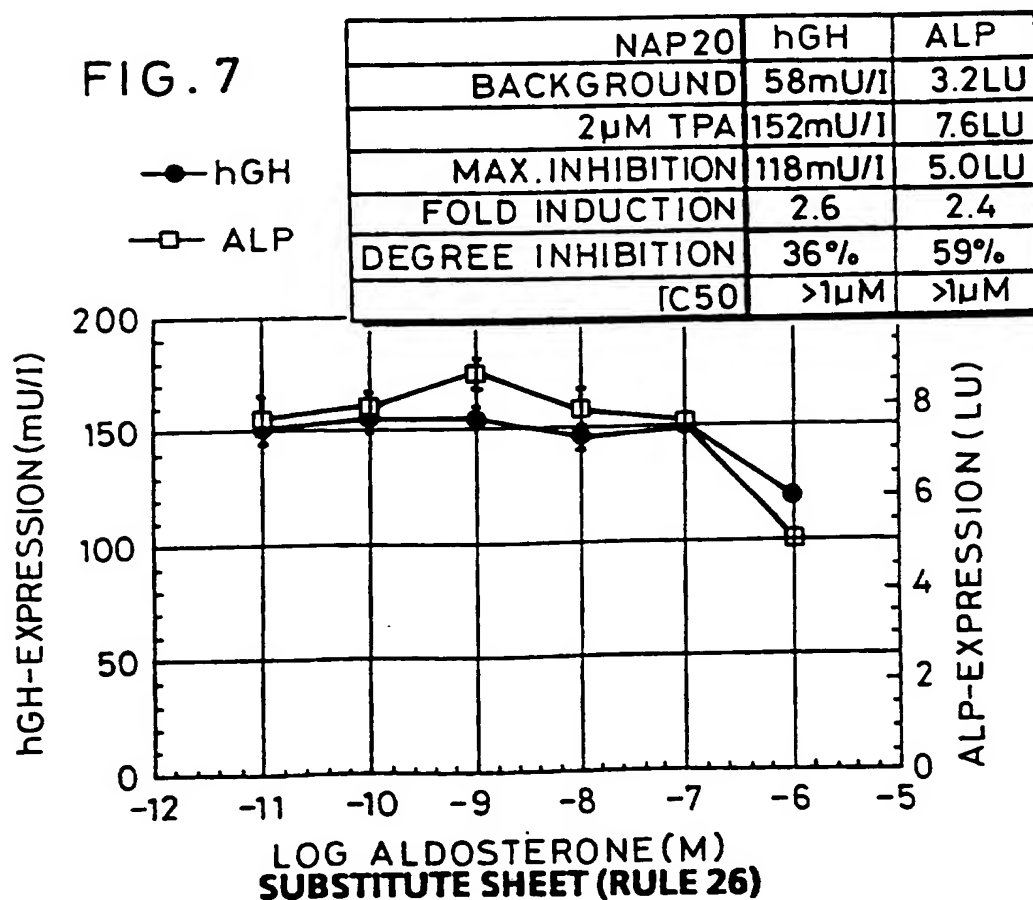


FIG. 7





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FIG. 8

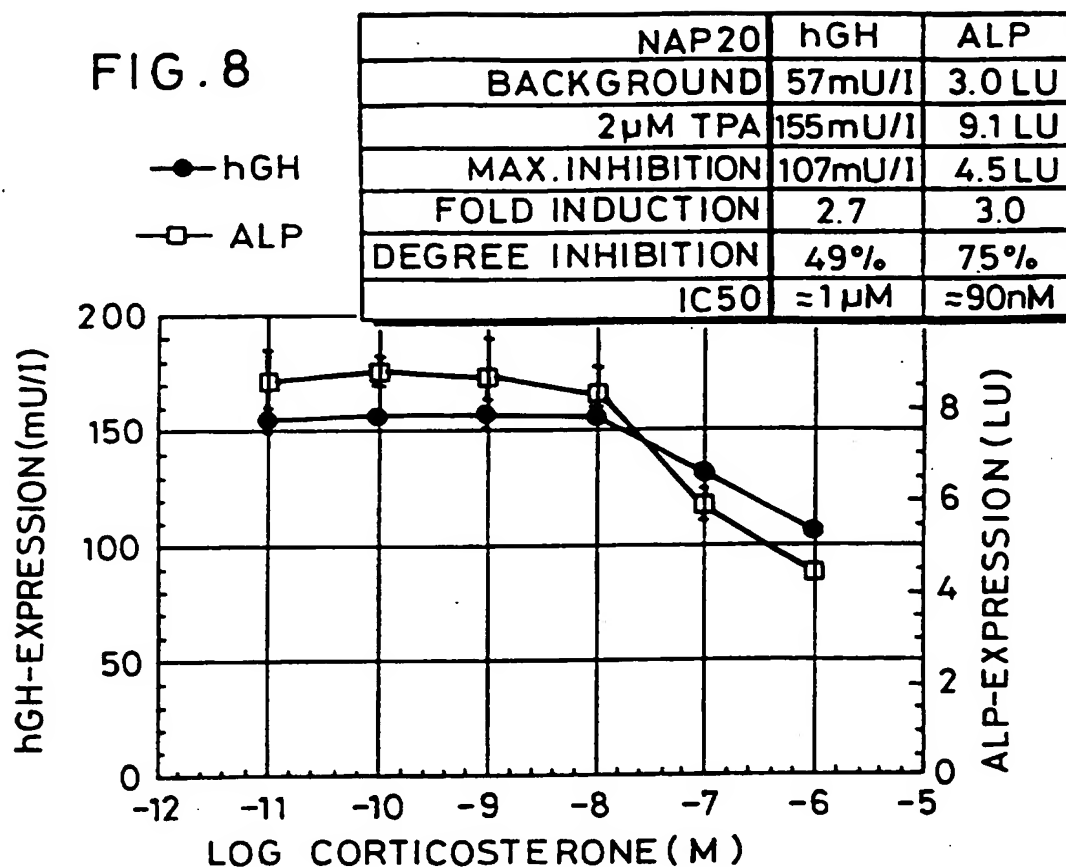
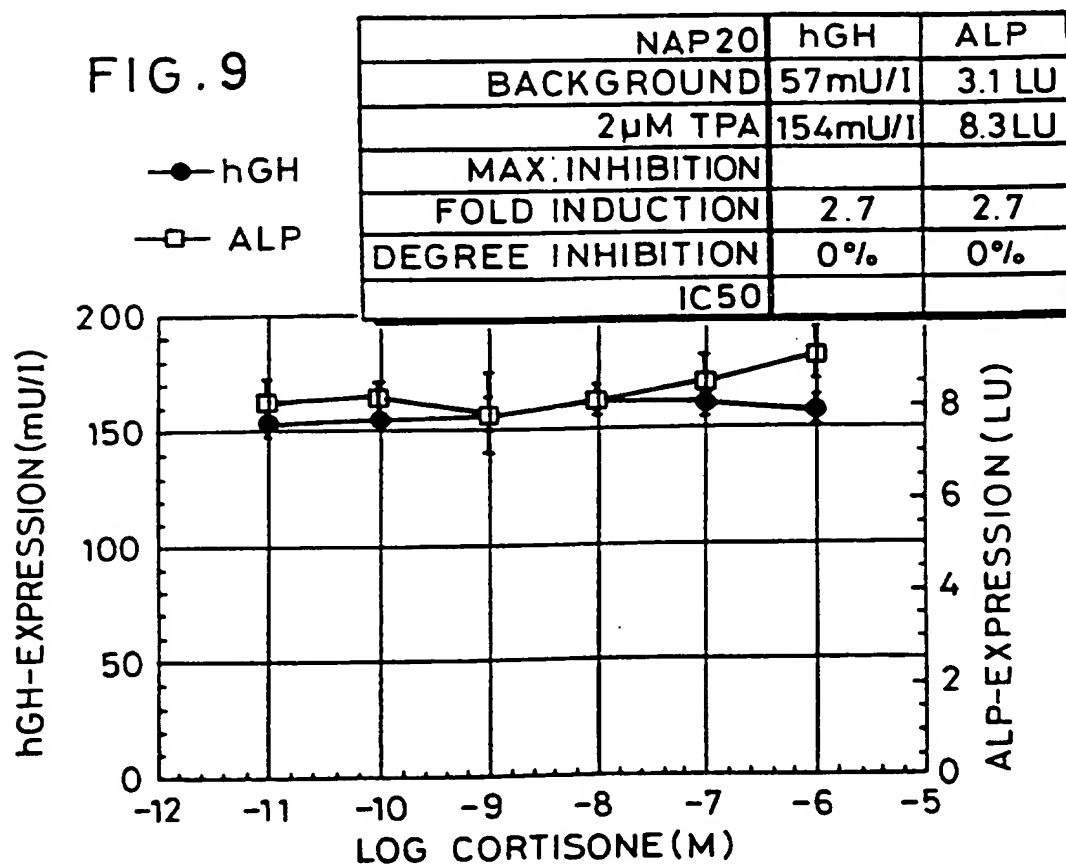


FIG. 9



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FIG. 10

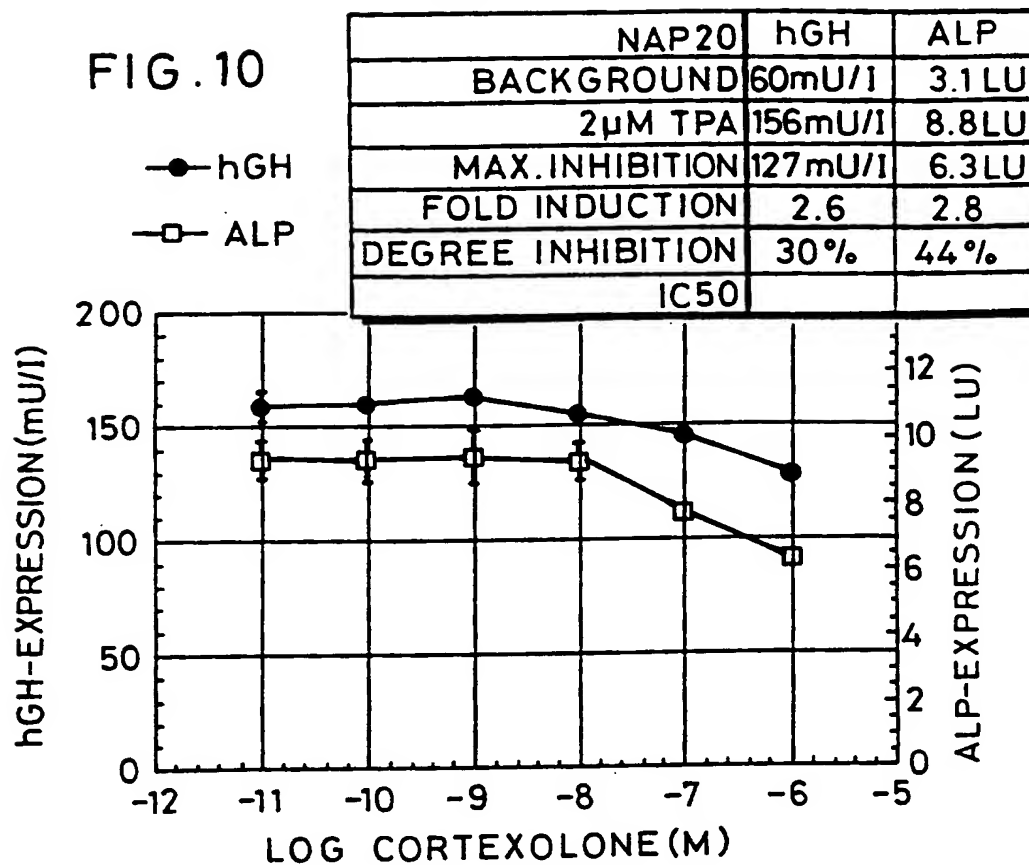
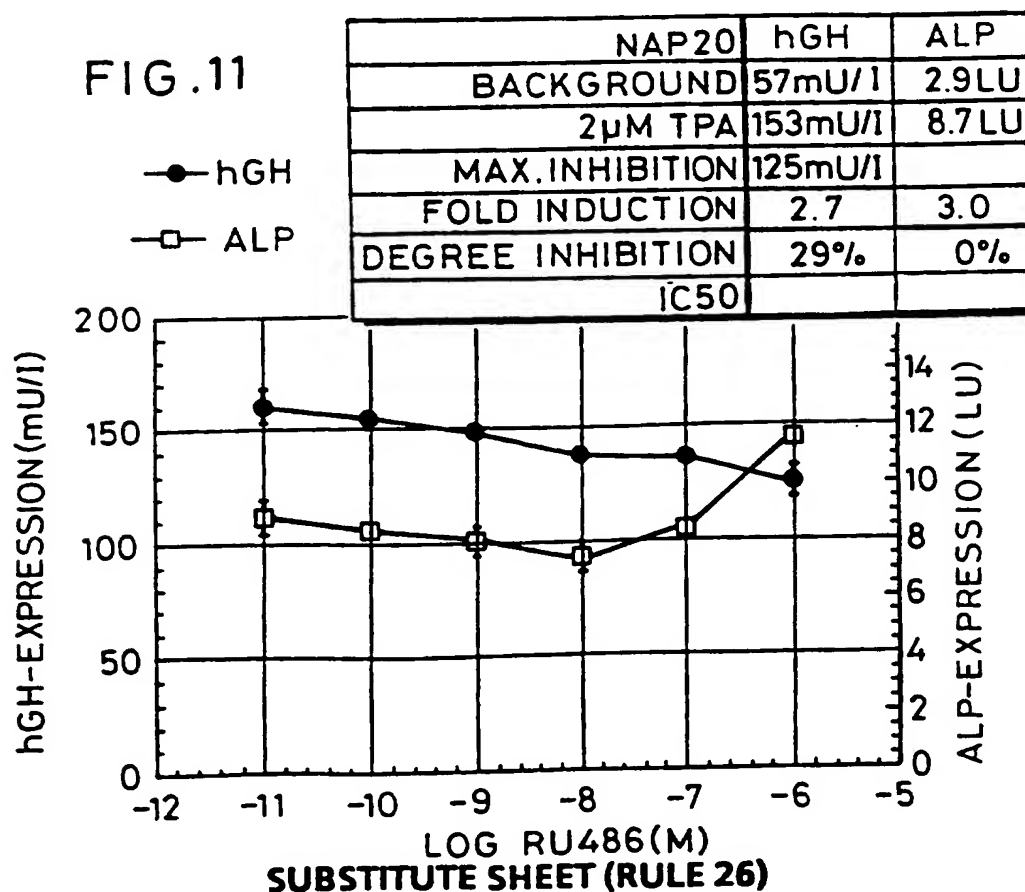


FIG. 11





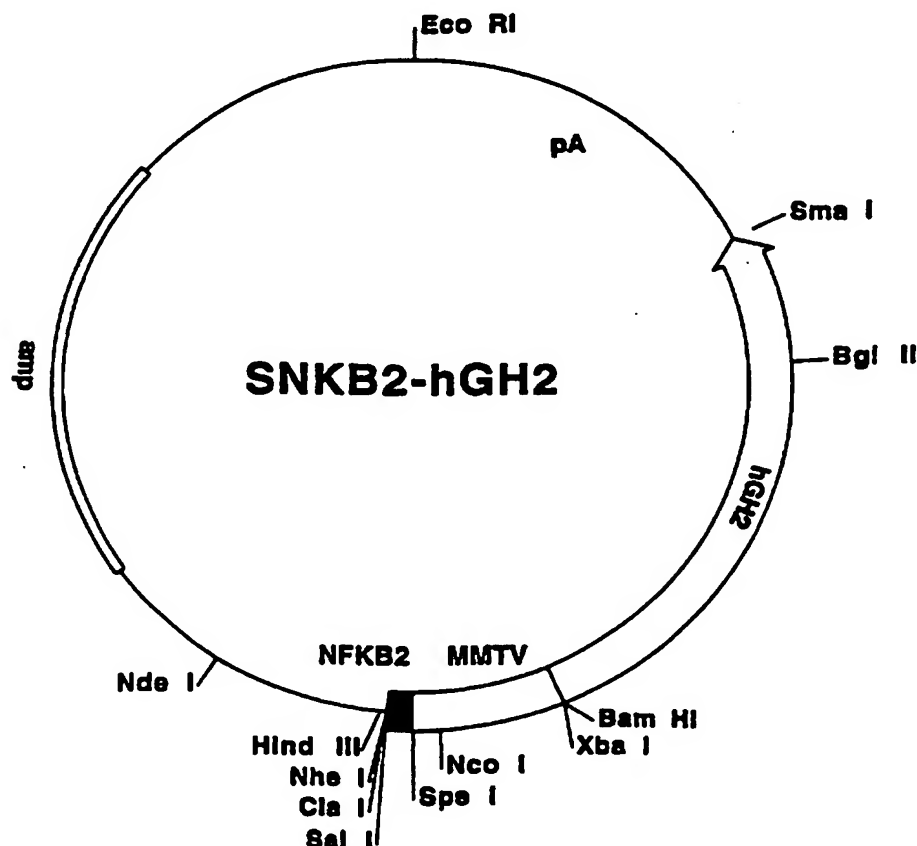
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(51) International Patent Classification <sup>6</sup> : <b>C12N 5/10, 15/67, 15/85, C12Q 1/68, C07K 14/61</b>		A3	(11) International Publication Number: <b>WO 96/17927</b>
			(43) International Publication Date: 13 June 1996 (13.06.96)
(21) International Application Number: <b>PCT/EP95/04819</b>		(81) Designated States: AU, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(22) International Filing Date: 5 December 1995 (05.12.95)		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(30) Priority Data: 9424497.7      5 December 1994 (05.12.94)      GB		(88) Date of publication of the international search report: 29 August 1996 (29.08.96)	
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(54) Title: REPORTER CELL LINE

## (57) Abstract

The present invention provides a cell line including a first reporter gene arranged to express an assayable first gene product, expression of the first reporter gene being AP-1 mediated, and a second reporter gene arranged to express a second assayable gene product, expression of the second reporter gene being mediated by NF $\kappa$ B. Thus the present invention provides a convenient one cell line-based assay system which can be used to rapidly test a large number of compounds for potential anti-inflammatory activity. According to a further aspect of the invention there is provided a method of testing a compound for both AP1 and NF $\kappa$ B inhibitory activity, the method comprising providing cells in accordance with the first aspect of the invention, stimulating AP1 and NF $\kappa$ B activity in the cells and contacting the cells with the compound to be tested and monitoring expression of the first and second gene products wherein inhibition of AP1 expression of the first gene product, and inhibition of NF $\kappa$ B mediated expression of the second gene product is indicative that the compound has both AP1 and NF $\kappa$ B-inhibitory activity.



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## INTERNATIONAL SEARCH REPORT

Inter national Application No

PC 1/EP 95/04819

A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 6 C12N5/10 C12N15/67 C12N15/85 C12Q1/68 C07K14/61

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Date of the actual completion of the international search

5 June 1996

Date of mailing of the international search report

27.06.96

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